

**Amendments to the Specification:**

Please replace the paragraph on page 28, lines 16-19, with the following amended paragraph:

- iii) Use as a marker in cell or organelle fusion. By labelling one or more cells or organelles with the novel proteins, for example, F64L-S175G-E222G-GFP, and other cells or organelles with same or another fluor, ~~fusions~~ cell/organelle fusion events such as heterokaryon formation can be monitored.

Please replace the paragraph on page 34, line 23 through page 35, line 15, with the following amended paragraph:

The gene for the mutant F64L-S175G-E222G-GFP (Example 2) was excised from pTARGET with *Bam*HI and *Sal*I and sub-cloned into the IPTG-inducible, GST-fusion vector pGEX-6P1 (Amersham Pharmacia Biotech). *E. coli* JM109 cells (Promega) containing an expression vector with the GST-GFP gene fusion were grown at 30°C to an OD<sub>600</sub>=0.6 in 2x YT broth containing 100 µg/ml ampicillin. Protein expression was induced with IPTG (0.1 mM) and incubation continued for 16 hours. Cells were pelleted by centrifugation, resuspended in PBS and lysed by sonication (four 10 second bursts at

20 µm with intermittent cooling on ice). Cellular debris was removed by centrifugation and the lysate containing soluble GST-GFP fusion protein was purified using glutathione sepharose columns (Amersham Pharmacia Biotech). Protein was then exchanged and eluted in PBS using a PD10 column (Amersham Pharmacia Biotech). The presence of a single band of correct molecular weight in the protein preparation was confirmed by SDS-PAGE using 4-12% ~~Bis-Tris~~ Bis-TRIS(Tris Hydroxymethylaminoethane)-HCl buffered polyacrylamide gel sold under the trademark NuPAGE-gel electrophoresis (by Invitrogen). To assess protein concentration and purity, the protein preparation was subjected, in duplicate, to acid hydrolysis and filtration before amino acid analysis by ion exchange chromatography using a Pharmacia alpha plus series II analyser.

Please replace the paragraph on page 35, line 17 through page 36, line 2, with the following amended paragraph:

The extinction coefficient (Table 2) was determined on a UV/vis spectrometer (Unicam). Quantum yield (Table 2) was determined according to the method documented by Patterson et al (Biophysical Journal, (1997), 73, 2782-2790). Samples of equal optical density at respective absorbance maxima were prepared, and diluted, in 10mM ~~Tris-HCl~~ TRIS(Tris Hydroxymethylaminoethane).HCl pH 8 for the purified GFP preparation and a fluorescein reference standard (Molecular Probes). Fluorescence emission was measured in the region 490 – 600nm using a LS50B luminescence

spectrometer (Perkin Elmer) and results for the GFP preparation were compared directly to those for the fluorescein standard (QY=0.85).

Please replace the paragraph on page 36, lines 8-17, with the following amended paragraph:

To evaluate the degree of photodegradation of the mutants F64L-S175G-E222G-GFP and F64L-E222G relative to wtGFP, 50ng of DNA was transfected into HeLa cells according to the method outlined in Example 3. For 50-80% confluency on the day of transfection, HeLa cells were plated at a density of  $5 \times 10^3$ /well in a ~~ViewPlate~~<sup>TM</sup> 96-well clear bottom, sterile and tissue culture treated microplate sold under the trademark VIEWPLATE (Packard, Meriden CT, USA). Twenty-four hours after transfection, the cells were imaged live on a ~~LEADseeker~~<sup>TM</sup> ~~Cell Analysis System~~ CCD-based multimodality imaging instrument sold under the trademark LEADSEEKER (Amersham Pharmacia Biotech) and bleached at high laser power (19.94mW) with a 488nm Argon laser (emission filter 535-45nm). Thirty-two individual images were taken over 260s with non-continuous illumination and all fluorescent proteins showed marked photodegradation as shown in Figure 6.

Please replace the paragraph on page 37, line 18 through page 38, line 5, with the following amended paragraph:

CHO-hir, P65-GFP cells were seeded into 96 well microtitre plates at a confluency of  $5 \times 10^3$  cells/well in DMEM media (Sigma) containing penicillin/streptomycin, L-glutamine (GibcoBRL) and incubated overnight at 37 °C. 1 hr before the assay was run, the media was removed and replaced with 100 µl serum free DMEM/well. 100 µl of 5 µM DRAQ5 (Biostatus) in Krebs buffer was added to each well and incubated for 15 minutes at 37°C. The plate was then placed in the ~~imager~~ (LEADseeker Cell Analysis System) CCD-based multimodality imaging instrument sold under the trademark LEADSEEKER and wells were imaged at varying time points following addition of agonist (50µl of 40 ng/ml IL1β). Translocation of the P65-GFP was observed from the cytoplasm to the nucleus following agonist addition. The ratio of nuclear/cytoplasmic fluorescence is shown in Figure 7.